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Value of Coxsackie and Adenovirus Receptor (CAR) Gene Expression in Colorectal Cancer

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ABSTRACT

Background: The Coxsackie virus and Adenovirus Receptor (CAR) is a cellular protein that has a role in cell adhesion, signaling, and viral infection. There is much disagreement over the significance of CAR expression in colorectal carcinoma development, with some research suggesting CAR downregulation and others indicating that CAR enabled complicated effects during colorectal carcinogenesis.

Objective: This study aimed to elucidate the difference in CAR expression levels in colorectal cancer (CRC) tissue versus normal colon tissue, and to correlate the expression levels with the disease stage.

Patients and methods: Fifty patients with proven colorectal cancer were enrolled in this study. During surgical excision treatment, 50 pairs of CRC tissue and normal tissue samples were obtained and examined for CAR expression levels using reverse transcriptase Real time PCR.

Results: CRC specimens showed significantly downregulated CAR gene expression when compared to nearby safety margin specimens. No significant differences were found in CAR gene expression levels in CRC tissue based on patients' gender, tumor site, size, associated LN metastasis and tumor stage ($p > 0.05$ for each). However, stratifying cases into early (stages I and II) and advanced (stages III and IV) revealed that lower CAR gene expression was significantly associated with advanced CRC stages.

Conclusion: Low CAR gene expression may have a potential role in colorectal carcinogenesis and its level is associated with advanced CRC stages with poorer prognosis.

Keywords: Adenovirus receptor, Colonic neoplasms, Carcinogenesis, Coxsackie virus infections.

INTRODUCTION

The fourth most frequent cancer worldwide is colon cancer, while rectal cancer is the eighth most common cancer. In total, colorectal carcinomas (CRCs) rank the third among cancers diagnosed worldwide [(1)]. CRC often develops when certain epithelial cells undergo a number of genetic or epigenetic changes [(2)]. Risk factors of CRC include older age, excessive alcohol use, limited physical activity, obesity, imbalanced diet, a family history of polyps, and inflammatory bowel disease [(3)].

Metastasis usually occurs in around 30–50% of individuals after surgical treatment of localized tumor, whereas 25% of patients directly present with metastatic disease [(4)]. The local tumor growth, as well as the existence of regional and distant metastases, are the most important factors in determining the prognosis of colon cancer [(5)].

On the surface of epithelial cells, the transmembrane glycoprotein known as the CAR was initially identified as a location for viral attachment [(6)]. Furthermore, it was recognized as a part of the tight junction complex [(7)]. It is a member of the immunoglobulin-like surface molecule subfamily and seems to be involved in cell adhesion or intercellular recognition [(8)]. Numerous solid tumours, such as those of the ovaries, lungs, breast, and bladder, have been shown to have reduced CAR expression. These include cancers with poor differentiation and late disease stages [(9-11)].

On the contrary, CAR has been hypothesized to promote the growth of adenocarcinomas due to the presence of high CAR expression in early-stage breast and esophageal cancers [(12)]. Additionally, CAR has been demonstrated to prevent apoptosis in adenocarcinoma cells and is essential for optimal tumor cell proliferation [(13)].

There is a strong controversy in the role of CAR expression in colorectal carcinoma, with some studies demonstrating CAR downregulation [(14-15)]. However, others suggest that CAR expression, maybe through its stage-dependent subcellular localization, causes complicated consequences throughout colorectal carcinogenesis [(11)].

Therefore, in this study we aimed to elucidate the difference in CAR expression levels in CRC tissue versus normal colon tissue, also to correlate the expression levels with the disease stage.

MATERIAL AND METHODS

Study Design and Participants: This is a cross-sectional case-control study, which was carried out in Gastroenterology Surgical Center and Clinical Pathology Department, Mansoura University through the period from July 2020 to March 2022 after approval from ethics

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and research committee of Mansoura University hospital. The subjects enrolled in this study were 50 colorectal cancer patients. The diagnosis of CRC was based on clinical, laboratory and radiological imaging (abdominal ultrasound, abdominal Multislice CT scan) and confirmed by post-surgical resection histopathological evaluation to confirm diagnosis and provide grading and staging.

Inclusion Criteria: 1. Patients with confirmed colorectal cancer (CRC): patients who had a confirmed diagnosis of CRC based on histopathological evaluation. **2.** Adults aged 18 to 80 years. This age range reflects the typical age group affected by CRC. **3.** Both male and female participants were included to ensure a diverse study population.

Exclusion Criteria: 1. Inflammatory Bowel Diseases: Patients with a history of inflammatory bowel diseases (e.g., Crohn's disease or ulcerative colitis) as these conditions can influence gene expression and may confound the results. **2.** Other Malignancies: Patients with a concurrent diagnosis of other malignancies to maintain the focus on colorectal cancer. **3.** Previous CRC Treatment (e.g. surgery, chemotherapy or radiation therapy) to avoid potential treatment-related effects on CAR gene expression. **4.** Inability to provide informed consent. **5.** Insufficient tissue samples for CAR gene expression analysis.

Sample Acquisition: During surgical excision treatment, 50 pairs of CRC tissue (each around 1 gm) and normal tissue (beyond the safety margin by 1-2 cm from the tumor edge). All samples were stored in saline at -70 °C.

Quality Assessment and bias mitigation: To ensure the quality of our study and mitigate the risk of bias, we followed rigorous protocols for sample collection, storage, and analysis. All laboratory procedures were performed by trained personnel following standardized operating procedures, and quality control measures, which were strictly adhered to during data acquisition.

Immunohistochemical testing: Immunohistochemical testing using primary antibodies against coxsackie virus/adenovirus receptor was conducted on formalin-fixed tissue using a Ventana BenchMark XT automated stainer (Ventana, Tucson, AZ, USA).

RT- Real time PCR for CAR gene expression: A tissue size (50 mg) was used for RNA extraction with TRIzol Lysis Reagent with the manufactural guide (Qiagen, Germany). In 20-50 µl of RNase-free water, the RNA pellet was suspended. NanoDrop (Thermo Fisher Scientific, USA) was used to determine the concentration and purity of RNA. The A260/A280 ratio of pure RNA was 1.9-2.1. Single-stranded cDNAs were generated using the reverse Transcriptase RT Kit (Thermo Fisher Scientific, USA), according to the manufacturer's directions using the thermal cycler (Techne Genius, UK), that was programmed to incubate tubes for 10 min at 25 ºC then 120 min at 37ºC then incubation for 5 min at 85ºC to inactivate Multiscribe Reverse transcriptase enzyme**.**

PCR quantification was performed using miSCript primer assay and maxima SYBR Green qPCR Kit for PCR amplification (Thermo Fisher Scientific, USA). The primers for CAR gene and PGM1 housekeeping gene were supplied by Qiagen, Germany. The housekeeping gene was used as the endogenous control.

Primer sequences

- CAR gene primers: (CARf,5'-CGTGCTCCTGTGCGGAGTAGT-3';CARr,5'-GACCCATCCTTGCTCTGTGCT-3'). The length of the expected product was 1068 (α-transcript) or 806 (β-transcript) bp of CAR gene.
- Phosphoglucomutase-1 (PGM-1), human housekeeping gene primers: (PGMf,5'TCCGACTGAGCGGCACTGGGAGTGC-3';PGMr,5' GCCCGCAGGTCCTCTTTCCCTCACA-3'). The length of the expected product was 382 bp [(16)].

The template cDNA was adjusted to be less than 500 ng per reaction. The primer concentration adjusted to be 0.3 uM (0.05-0.9 uM). For each sample two tubes were included, one contained the CAR primers and the other contained the house keeping genes primers. The real time PCR assay was performed by applied biosystem step one ™ Real-Time PCR system (Thermo Fisher Scientific, USA).

Interpretation of the result:

Amplified products were loaded on 2% agarose gel and electrophoresis was carried for 30 min at 100 volts to ensure specificity of the products. The data were collected from the software in the form of Ct sample and Ct HK gene. For gene expression quantification, the Ct technique of comparison was utilised. First, the expression levels of each gene that codes for a housekeeping enzyme in a sample were normalised to the expression level of that gene (△Ct). Relative gene expression levels were calculated using the $2^{-\triangle\triangle CT}$ technique to analyse the results.

Where ΔΔ (Ct) = ΔCt patient - mean ΔCt of control subjects.

ΔCt patient = Ct value of target – Ct value of HK.

Outcomes definition: the primary outcome was defined as the CAR gene expression level in CRC tissue compared to normal colon tissue. Secondary outcomes included the association between CAR expression and clinical

parameters such as disease stage, tumor size, gender, and other relevant factors.

Ethical approval: Mansoura Medical Ethics Committee of Mansoura Faculty of Medicine gave its approval to this study. All participants gave written consent after receiving all information. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis

SPSS V. 25.0 was used to edit, code, and tabulate the gathered data. The normality of the data distribution was examined using the Shapiro-Wilk test.

A relevant analysis was conducted based on the data type collected for each parameter, after the data were provided. Relative percentages and frequencies were used to display the qualitative data. Two independent groups of normally distributed variables (parametric data) were compared using the independent samples t-test. The fixed P value for statistical significance was set at 0.05, and for a very significant result, it was ≤ 0.001.

RESULTS

Fifty CRC patients participated in the current study. Their mean age was 57.9 ± 11.6 years, they were 32 males (64%) and 18 females (36%). Only 7 (14%) were smokers, 5 (10%) had ischemic heart disease, 10 (20%) were diabetics, 9 (18%) were hypertensive and 20 (40%) had previous abdominal surgeries. Abdominal pain was the most common symptom in 41 (82%), followed by bleeding per rectum or disturbed bowel habits in 17 (34%), weight loss in 9 (18%), and nausea and vomiting in 6 (12%) patients (Table 1).

Table (1): Tumor features in all studied cases

Size (cm)		Median, range	5	1-13
Size	**≤5cm**	**N, %**	28	56%
	>5cm	**N, %**	22	44%
Differentiation	**Moderate**	**N, %**	50	100%
Site	**Colon**	**N, %**	39	78%
	Rectum	**N, %**	11	22%
LN metastasis		**N, %**	11	22%
Distant metastasis		**N, %**	2	4%
Stage	**I**	**N, %**	4	8%
	II	**N, %**	27	54%
	III	**N, %**	17	34%
	IV	**N, %**	2	4%

Colorectal specimens showed significantly downregulated CAR gene expression when compared to nearby safety margin specimens (Table 2).

Table (2): Comparison of CAR gene expression level between healthy and cancerous tissues

	Healthy tissue N=50		CRC tissue N=50		p
	Median	Range	Median	range	
CAR gene expression	0.829	0.013-28.840	0.467	0.004-4.691	0.018

ROC curve of CAR gene expression level was conducted for discrimination between healthy and cancerous tissues. CAR gene expression level showed AUC of 0.590 at cut off value of 1.77 carrying sensitivity of 80%, specificity of 38%, PPV of 56.3%, NPV of 65.5%, and an accuracy of 59% (Figure 1).

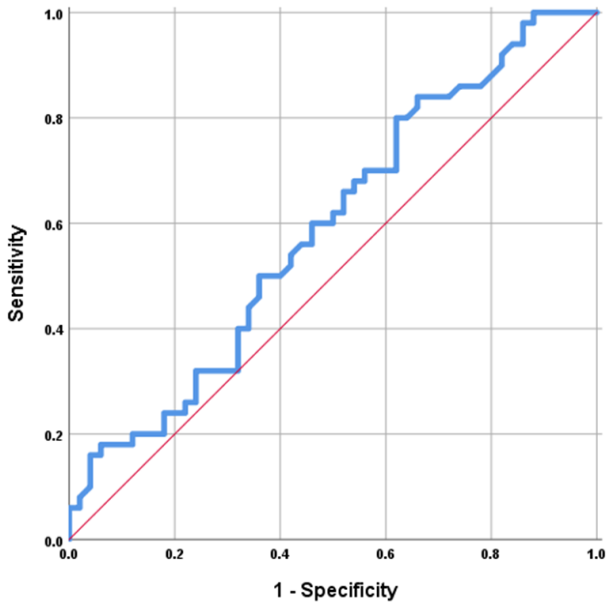

Figure (1): ROC curve of CAR gene expression level for discrimination between healthy and cancerous tissues.

In table (3), there were no significant differences in the level of CAR gene expression based on the patients' gender, tumor site, size, associated LN metastasis, and tumor stage (p>0.05 for each). However, stratifying cases into early (stages I and II) and advanced (stages III and IV) revealed that lower CAR gene expression was associated with advanced CRC stages with a significant negative correlation (Figure 2). Lower CAR gene expression was linked to more advanced CRC stages (stages III & IV) compared to earlier stages (stages I & II) with a significant negative correlation.

Table (3): Comparison of CAR gene expression level in CRC tissue according to other studied parameters

		CAR gene expression in CRC tissue			
		Median	Minimum	Maximum	P
Gender	Male	0.423	0.005	4.691	0.976
	Female	0.579	0.004	2.908	
Size	≤5cm	0.527	0.009	4.691	0.725
	>5cm	0.423	0.004	3.294	
Site	Colon	0.590	0.009	4.691	0.128
	Rectum	0.202	0.004	1.625	
LN metastasis	Absent	0.547	0.004	4.691	0.386
	Present	0.202	0.038	2.809	
Stage	I	1.138	0.095	2.828	0.141
	II	0.551	0.009	4.691	
	III	0.238	0.004	1.591	
	IV	0.584	0.202	0.966	
Stage	I+II	0.651	0.009	4.691	0.025*
	III+IV	0.238	0.004	1.591	

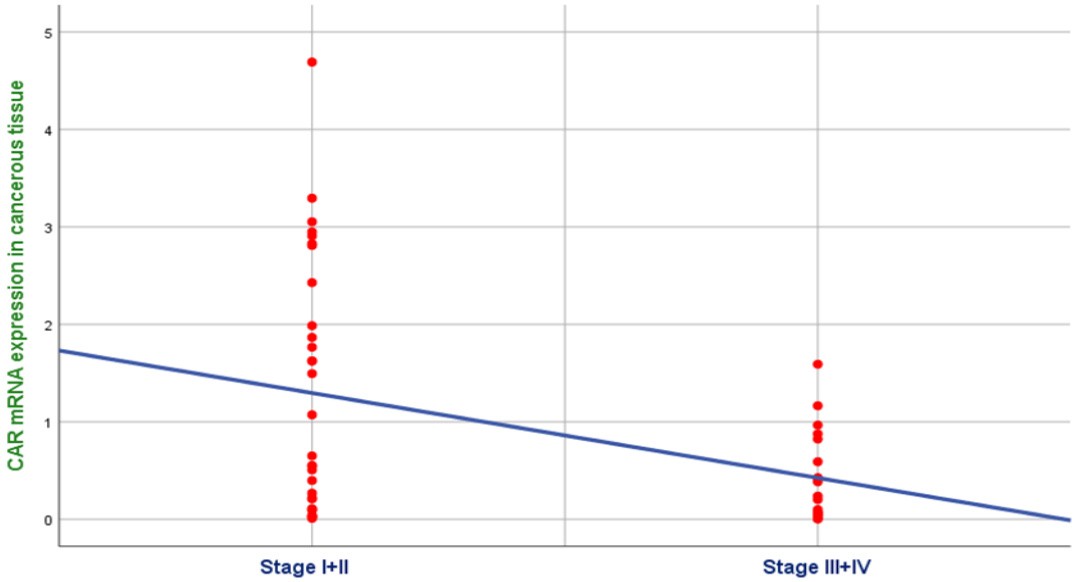

Figure (2): The Correlation between CAR gene expression in CRC tissue and tumor stage.

Logistic regression analysis was conducted for prediction of CRC advanced staging using age, gender, smoking, CEA, CA19-9. CAR gene expression level in healthy and cancerous tissues as confounders. Lower CAR gene expression in cancerous tissue was suggested to be risk predictor for CRC advanced stage (Table 4).

Table (4): Regression analysis for prediction of advanced stages of CRC

	P	OR	95% CI
Age	0.983	1.002	0.964-1.036
Gender	0.832	0.915	0.402-2.082
Smoking	0.377	1.595	0.566-4.494
CEA	0.430	1.003	0.996-1.009
CA19-9	0.468	0.999	0.997-1.002
CAR gene expression in healthy tissue	0.184	1.064	0.992-1.142
CAR gene expression in CRC tissue	**0.021**	0.415	0.197-0.874

*OR: odds ratio; CI, confidence interval.

DISCUSSION

Globally, colorectal carcinoma ranks third in frequency of cancer cases. In 2020, there were around 1.9 million new cases. Despite this enormous number, a conclusive cure has not been discovered [17]. Depending on whether virus targets tumour cells, oncolytic virus therapy is a potential treatment for a variety of cancers [18]. CAR is one of the most widely used viral vectors and was found to be expressed in many malignancies [19]. The expression level of CAR protein is variable; upregulation of CAR was found in ovarian, cervical, and lung cancers, while downregulation was reported in parotid, kidney, and colorectal carcinomas [15].

The downregulation of CAR gene expression in the present study is in agreement with **Reeh *et al.*** [15] who demonstrated decreased CAR gene expression in CRC. Similarly, **Ma *et al.*** [20] showed that 240 out of 251 (95.6%) noncancerous colorectal mucosa samples had positive expression of CAR protein; this number was much higher than that of CRC (40.6%). A significant degree of heterogeneity in CAR expression levels was also noted by **Zhang *et al.*** [14] who found that CAR downregulation was present in almost 75% of the cases. Therefore, it was proposed that CAR expression reduction encourages primary CRC development and metastasis [21].

CAR upregulation, on the other hand, has been reported in several malignancies, including endometrial, ovarian, cervical, breast, and lung cancers, as well as neuroblastomas and medulloblastomas [22-28]. Moreover, it has been linked to poorer prognosis in breast and lung cancers [10, 24].

No significant differences were found in CAR gene expression level in CRC tissue based on patients' gender, tumor site, size, associated LN metastasis and tumor stage ($p>0.05$ for each). However, **Zhang *et al.*** [14] showed that rather than lymph node involvement, the size of the original tumour was correlated with CAR expression. CAR expression frequently declined in tumours with a diameter greater than 5 cm, suggesting that CAR downregulation in colon carcinomas may be linked to the development of the tumour. Additionally, there was a relationship between age and CAR expression. Patients with lesser CAR expression were more likely to be under 50 years old. Contrary to our results, CAR immunopositivity was reported by **Korn *et al.*** [29] in 60% of CRC patients with liver metastases. **Rauen *et al.*** [30] also showed that, in comparison with primary disease, CAR immunopositivity was much greater in metastatic prostate cancer.

In our study, Lower CAR gene expression was significantly related to advanced CRC stages. In the same line, **Reeh *et al.*** [15] found that various early phases of malignant transformation, such as non-invasive bladder cancer, thyroid adenoma, and basalioma, have widespread CAR expression. CAR expression was greater in endometrial and hepatocellular carcinomas in their late stages than in their early stages. On the other hand, low levels of CAR expression were discovered in colon cancer, Merkel cell carcinoma, prostate cancer, and several forms of breast cancer [15].

Prostate carcinoma showed a decrease in CAR expression in the main tumour but an increase in bone metastases. In bladder cancer, CAR expression was linked with clinical stage, pathologic grade, lymph node status, and survival. The prognostic significance of CAR remains unclear, despite the possibility that its expression plays a role in tumour growth [31].

On the horizon of cancer treatment, oncolytic adenovirus therapy holds a great promise. The key to effectively targeting tumor cells with oncolytic adenoviruses lies in the presence of adenovirus receptors on these cells. It's important to note that various adenovirus types attach to distinct receptors on the cell surface, and the expression of these receptors can vary across different types of tumors [32]. The present study revealed that CAR gene expression level could discriminate between healthy and cancerous tissues.

Limitations: The current study's limitations included small sample size. Moreover, the expression level of CAR in relation to the degree of differentiation was not evident since all the cases fulfilling the inclusion criteria were moderately differentiated. In future studies, we intend to increase the sample size to enhance the statistical power and provide more comprehensive insights into the role of CAR expression in colorectal cancer.

CONCLUSION

In conclusion, the expression of CAR gene was significantly downregulated in colorectal cancer and its level was associated with more advanced stages of the disease and a poorer prognosis for patients. This study also provided an evidence for the role of CAR gene in the development and progression of colorectal cancer. However, to validate these results and investigate the possible application of the CAR gene as a therapeutic target in the treatment of colorectal cancer, more research is necessary.

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Competing interests: Nil.

REFERENCES

1. **Bray F, Ferlay J, Soerjomataram I *et al.* (2018):** Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: Cancer Journal for Clinicians, 68 (6): 394-424.
2. **Ewing I, Hurley J, Josephides E *et al.* (2014):** The molecular genetics of colorectal cancer. Frontline Gastroenterology, 5 (1): 26-30.
3. **Gu M, Huang Q, Bao C *et al.* (2018):** Attributable causes of colorectal cancer in China. BMC Cancer, 18 (1): 1-9.
4. **Salvatore L, Aprile G, Arnoldi E *et al.* (2017):** Management of metastatic colorectal cancer patients:

guidelines of the Italian Medical Oncology Association (AIOM). ESMO Open, 2 (1): e000147. doi: 10.1136/esmoopen-2016-000147.

5. **Wong T, Chay W, Tan M *et al.* (2019):** Reproductive factors, obesity and risk of colorectal cancer in a cohort of Asian women. Cancer Epidemiology, 58: 33-43.
6. **Bergelson J, Krithivas A, Celi L *et al.* (1998):** The murine CAR homolog is a receptor for coxsackie B viruses and adenoviruses. Journal of Virology, 72 (1): 415-19.
7. **Raschperger E, Thyberg J, Pettersson S *et al.* (2006):** The coxsackie-and adenovirus receptor (CAR) is an in vivo marker for epithelial tight junctions, with a potential role in regulating permeability and tissue homeostasis. Experimental Cell Research, 312 (9): 1566-80.
8. **Cohen C, Shieh J, Pickles R *et al.* (2001):** The coxsackievirus and adenovirus receptor is a transmembrane component of the tight junction. Proceedings of the National Academy of Sciences, 98 (26): 15191-96.
9. **Yamashita M, Ino A, Kawabata K *et al.* (2007):** Expression of coxsackie and adenovirus receptor reduces the lung metastatic potential of murine tumor cells. International Journal of Cancer, 121 (8): 1690-96.
10. **Wunder T, Schmid K, Wicklein D *et al.* (2013):** Expression of the coxsackie adenovirus receptor in neuroendocrine lung cancers and its implications for oncolytic adenoviral infection. Cancer Gene Therapy, 20 (1): 25-32.
11. **Stecker K, Koschel A, Wiedenmann B *et al.* (2009):** Loss of Coxsackie and adenovirus receptor downregulates α-catenin expression. British Journal of Cancer, 101 (9): 1574-79.
12. **Küster K, Grötzinger C, Koschel A *et al.* (2010):** Sodium butyrate increases expression of the coxsackie and adenovirus receptor in colon cancer cells. Cancer Investigation, 28 (3): 268-74.
13. **Brüning A, Runnebaum I (2003):** CAR is a cell–cell adhesion protein in human cancer cells and is expressionally modulated by dexamethasone, TNFα, and TGFβ. Gene Therapy, 10 (3): 198-205.
14. **Zhang N, Song L, Wu X *et al.* (2008):** Proteasome inhibitor MG-132 modifies coxsackie and adenovirus receptor expression in colon cancer cell line lovo. Cell Cycle, 7 (7): 925-33.
15. **Reeh M, Bockhorn M, Görgens D *et al.* (2013):** Presence of the coxsackievirus and adenovirus receptor (CAR) in human neoplasms: a multitumour array analysis. British Journal of Cancer, 109 (7): 1848-58.
16. **Abdolazimi Y, Mojarrad M, Pedram M *et al.* (2007):** Analysis of the expression of coxsackievirus and adenovirus receptor in five colon cancer cell lines. World J Gastroenterol., 13 (47): 6365-69.
17. **Kelly E, Russell S (2007):** History of oncolytic viruses: genesis to genetic engineering. Molecular Therapy, 15 (4): 651-59.
18. **Bergelson J, Cunningham J, Droguett G *et al.* (1997):** Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. Science, 275 (5304): 1320-23.
19. **Arnberg N (2012):** Adenovirus receptors: implications for targeting of viral vectors. Trends in Pharmacological Sciences, 33 (8): 442-48.
20. **Ma Y, Wang X, Han Y *et al.* (2016):** Loss of coxsackie and adenovirus receptor expression in human colorectal cancer: A potential impact on the efficacy of adenovirus-mediated gene therapy in Chinese Han population. Molecular Medicine Reports, 14 (3): 2541-47.
21. **Stecker K, Vieth M, Koschel A *et al.* (2011):** Impact of the coxsackievirus and adenovirus receptor on the adenoma–carcinoma sequence of colon cancer. British Journal of Cancer, 104 (9): 1426-33.
22. **Dietel M, Häfner N, Jansen L *et al.* (2011):** Novel splice variant CAR 4/6 of the coxsackie adenovirus receptor is differentially expressed in cervical carcinogenesis. Journal of Molecular Medicine, 89 (6): 621-30.
23. **Giaginis C, Zarros A, Papaefthymiou M *et al.* (2008):** Coxsackievirus and adenovirus receptor expression in human endometrial adenocarcinoma: possible clinical implications. World Journal of Surgical Oncology, 6 (1): 1-8.
24. **Martin T, Watkins G, Jiang W (2005):** The Coxsackie-adenovirus receptor has elevated expression in human breast cancer. Clinical and Experimental Medicine, 5 (3): 122-28.
25. **Martino T, Petric M, Weingartl H *et al.* (2000):** The coxsackie-adenovirus receptor (CAR) is used by reference strains and clinical isolates representing all six serotypes of coxsackievirus group B and by swine vesicular disease virus. Virology, 271 (1): 99-108.
26. **Pearson A, Koch P, Atkinson N *et al.* (1999):** Factors limiting adenovirus-mediated gene transfer into human lung and pancreatic cancer cell lines. Clinical Cancer Research, 5 (12): 4208-13.
27. **Reimer D, Steppan I, Wiedemair A *et al.* (2007):** Soluble isoforms but not the transmembrane form of coxsackie-adenovirus receptor are of clinical relevance in epithelial ovarian cancer. International Journal of Cancer, 120 (12): 2568-75.
28. **Wang Y, Wang S, Bao Y *et al.* (2006):** Coxsackievirus and adenovirus receptor expression in non-malignant lung tissues and clinical lung cancers. Journal of Molecular Histology, 37 (3): 153-60.
29. **Korn W, Macal M, Christian C *et al.* (2006):** Expression of the coxsackievirus-and adenovirus receptor in gastrointestinal cancer correlates with tumor differentiation. Cancer Gene Therapy, 13 (8): 792-97.
30. **Rauen K, Sudilovsky D, Le J *et al.* (2002):** Expression of the coxsackie adenovirus receptor in normal prostate and in primary and metastatic prostate carcinoma: potential relevance to gene therapy. Cancer Research, 62 (13): 3812-18.
31. **Matsumoto K, Shariat S, Ayala G *et al.* (2005):** Loss of coxsackie and adenovirus receptor expression is associated with features of aggressive bladder cancer. Urology, 66 (2): 441-46.
32. **Hensen L, Hoeben R, Bots S (2020):** Adenovirus Receptor Expression in Cancer and Its Multifaceted Role in Oncolytic Adenovirus Therapy. Int J Mol Sci., 21 (18): 6828. doi: 10.3390/ijms21186828.